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(54) Title: <b>PROCESS FOR THE PRODUCTION OF COMBINATORIAL COMPOUND LIBRARIES</b>		
<p>(57) Abstract</p> <p>Described is a process for the production of a library from a plurality of different units consisting of a solid or semisolid carrier (bead), a synthetic ligand and a binary information structure (tag), by means of which the building blocks of the ligand are coded, and the use of that library for searching for novel classes of active ingredient and individual active ingredients. Also described are compounds found using that process. The process is characterized by the use of element atoms or ions as binary tags which are bound non covalently to the beads. Their unique combination which can be measured by e.g. mass spectrometry codes for the specific ligand.</p>		

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**Process for the production of combinatorial compound libraries**

The invention relates to a process for the production of a library of a plurality of different units consisting of a solid or semisolid carrier (bead), a synthetic ligand and a binary identification structure (tag) by means of which the building blocks of the ligand are coded; and to the use of that library for searching for novel classes of active ingredient and individual active ingredients. The invention relates also to compounds found using that process and to the use of those compounds as thrombin inhibitors.

There has been a steady increase recently in the demand for chemical compounds that bind selectively to a specific acceptor only. Those compounds can then be used, for example, as inhibitors, agonists or antagonists or for labelling. In that connection, the possibility has been developed of synthesising a multiplicity (a library) of different compounds simultaneously and of testing them for their ability to bind to a specific acceptor. Those libraries (combinatorial compound libraries, CCL) consist in general of natural amino acids or of nucleotides and, increasingly, also of modified amino acids, modified nucleotides and other chemical compounds that are used as building blocks.

The different individual ligands of those libraries are generally synthesised in parallel, either as mixtures or as physically separated individuals. That parallel synthesis allows libraries containing a very large number of different ligands to be produced within a relatively short period of time.

The literature describes *inter alia* two intrinsically different main strategies for the chemical synthesis of libraries consisting of a plurality of test candidates.

1. After synthesis the ligands are obtained together in solution (Houghten *et al.* Nature (1991) 354, 84-86).
2. The ligands are synthesised on a solid carrier, the synthesis being controlled in such a manner that only one species of ligand is bound per carrier (one bead one sequence; Lam *et al.* Nature (1991), 354, 82-84; Furka *et al.*, 14<sup>th</sup> Intern. Congr. Biochem. FR013, 1988).

With the first strategy there is the problem of identifying the desired ligands clearly, since all the ligands are present in the solution simultaneously. When identifying ligands having a number of variable positions, it is necessary to synthesise a pool for every possible building

block per variable position, with the result that, for example, in the case of a ligand consisting of four variable positions each of which can be occupied by one of 20 amino acids,  $4 \times 20 = 80$  pools have to be synthesised and tested.

The second strategy produces all the desired possibilities in a single pool and allows the solid carriers containing the desired ligands to be separated easily and hence the ligands to be identified. However the limitation in this case lies in the nature of the ligands that are used, since their composition must subsequently be determined. Thus the only ligands that are suitable therefor are those that can be clearly identified by sequencing (peptides of natural amino acids, DNA and RNA). A further disadvantage is the fact that the attachment of the ligands to solid carriers can lead to false-positive results, i.e. that some tests cannot be carried out with ligands that are bound to solid carriers.

The limitation of the second strategy to sequenceable ligands has often been circumvented by synthesising for each ligand a coding identification structure (tag). The disadvantage of that method is that under the reaction conditions required to synthesise the identification structure (tag) the ligand that is synthesised at the same time must remain stable, while the synthesis of the ligand must not alter the tag. That restricts the synthesis to strictly orthogonal synthesis, which greatly limits the choice of reactants.

In that method, the tag for identifying the ligand can be applied in the form of a sequenceable oligomer (WO-9306121) or in the form of a binary code (WO-9408051). In the case of binary codes, for example, various organic compounds are used which for analysis are then removed again chemically and identified individually (WO-9408051).

#### Summary of the invention

Surprisingly, it has now been possible to produce, without chemical reaction steps that would influence the ligand, a binary code by means of which the ligand synthesised on a bead can be identified clearly and with great sensitivity. Surprisingly, the binary code is simple to apply, is capable of coding a large number of different ligands and can be identified clearly and with a high degree of sensitivity in a very simple manner. In order to increase the accuracy of the identification, it is possible additionally to code each unit of the ligand clearly several times (redundant coding). Also surprising is the fact that despite the large number of washing steps carried out in the solid phase synthesis the elements or element salts applied remain bound in sufficient quantities for those elements or element salts to be identified at the end of the synthesis.

The possible building blocks of the ligands are described in the process according to the invention by a binary code (i.e. by the presence or absence of a specific element or element ion). Using that process,  $2^n$  possible building blocks can be coded using  $n$  elements or element ions. Thus using 30 different elements it is possible to code, for example, 1000 different building blocks, each in three different positions.

For example, a library for a ligand consisting of 3 building blocks with 5 possibilities for the first building block, 4 for the second building block and 5 for the third building block can be coded clearly by a set of 8 elements or element ions:

Position	Building block	Code
X	2-cyanobenzenesulfonyl chloride D-Phe N-benzylglycine $\beta$ -Ala acetyl	no element Pr La Sb + La Sb
Y	L-Pro D-Pro $\beta$ -Ala L-Asp	no element Nd Eu Nd + Eu
Z	L-Asp D-Arg $\beta$ -Ala L-Arg sarcosyl	no element Tb Ho Tb + Ho Lu

If, on analysis of a bead, the following elements or element ions are found

Pr, Nd, Tb and Ho,

this shows that the ligand consists of D-Phe-D-Pro-Arg. The combination

Pr and Tb

would code for D-Phe-Pro-D-Arg.

#### Detailed description of the invention

The subject of the invention therefore consists of a library of a plurality of different units each consisting of a solid or semisolid carrier, a synthetic ligand and an information structure (tag) by means of which the building blocks of the ligand can be identified, wherein

- a) each carrier unit carries only one type of ligand,
- b) each ligand is clearly identified by the presence or absence of one or more elements or element salts.

Typical libraries consist, for example, of more than 50 different ligands. In general, libraries of a far greater size, for example of  $\geq 1000$  or  $\geq 10^6$  different ligands, are synthesised.

Using the process according to the invention, it is possible, for example, to carry out two different tests:

- a) A first test for preliminary selection of ligands binding to the acceptor, the ligand being bound to the solid or semisolid carrier.
- b) A second test, after separation of the ligand from the carrier and the information structure, for further characterisation of the ligand. The second test can also examine the attachment of the ligand to the acceptor or may be a completely different test that cannot be carried out with bound ligands.

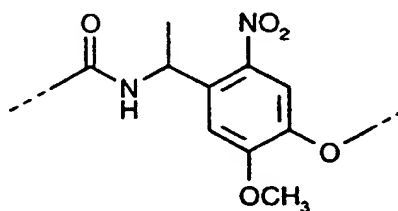
The term ligand includes any compound that can be synthesised selectively from a plurality of building blocks. The building blocks typically contain at least two reactive groups. Examples of such reactive groups are amino, azide, isocyanide, isocyanate, hydrazine, carbonyl, carboxyl, acyl-halogen, hydroxy, sulfhydryl, sulfonyl chloride, phosphate and halogen groups. In order to simplify the synthesis, the reactivity of those groups can be altered by means of protecting groups or activating groups. Examples of building blocks having two or more reactive groups are natural and non-natural amino acids,  $\omega$ -aminocarboxylic acids, saccharides, nucleotides and nucleotide analogues. When building blocks having more than two reactive groups are used, it is also possible to insert optional branches and cyclisations of the ligand. Building blocks having only one reactive group can be used as terminal groups.

Using the process according to the invention, which is described in detail below, it is also possible to produce libraries of ligands in which the individual building blocks are bound to a basic compound not only in series but also next to one another, in a specific configuration. For that purpose, for example, a basic building block containing several reactive groups is bound to the carrier either directly or *via* a linker and then individual building blocks, which in turn contain one or more reactive groups, are bound to that basic building block. Basic building blocks within the context of the invention are, for example, steroid basic structures, the basic structure of penicillin or penem, soraphenes, benzodiazepines, saccharides or desferrioxamine. Various reactive groups can then be fixed to those basic structures in various positions using standard chemical methods.

It is also possible to use ligands containing building blocks that cannot be identified by sequencing without further experimental effort, for example by means of known, automated methods.

Building blocks that cannot be clearly identified by sequencing are any chemical compounds apart from the 20 naturally occurring amino acids and the nucleotides that occur naturally in DNA and RNA. Examples are modified amino acids or nucleotides,  $\omega$ -aminocarboxylic acids, D-amino acids, saccharides, amino acids having saccharide side chains and terminal groups having only one reactive group, such as acetyl or benzyl.

The ligand is customarily bound to the solid or semisolid carrier *via* a linker. There may be used as linkers for the ligand, for example, chemical compounds having at least two reactive groups. Preference is given to linkers that tolerate both weakly basic and weakly acid conditions, which enables the ligands and the tag not only to be synthesised, but also to be tested, on the carrier. The preferred linkers can therefore be cleaved only by means of a specific reaction, for example methionine, which can be removed by cyanogen bromide, or are linkers that can be cleaved only under strongly basic conditions, under strongly acid conditions, by photolysis or under reducing or oxidising conditions, or using  $\text{Pd}^0$ . Special preference is given to linkers that form bonds that are cleavable under basic conditions but stable under acid conditions, which enables the ligand to be detached from the carrier selectively. Examples of linkers that can be used are p-hydroxymethylbenzoic acid, 4-hydroxymethylphenylacetic acid, benzhydrylamino, allyl, hydroxy-crotonyl-aminomethyl, 3-nitro-4-hydroxymethylbenzoic acid, p-nitrobenzhydrylamine or 4-[4,4'-bis(methylsulfinyl)-2-oxy-benzhydrylamino]butyric acid linkers, disulfide linkers, or linkers of the type having the following formula



each of which can in turn be bound *via* a group referred to in solid phase synthesis as a "handle".

It is also possible to use as linkers short peptides having a specific cleavage site for a protease, such as trypsin, ysc $\alpha$ , yscF or the V8-protease.

The information structure (tag) is formed by a combination (presence or absence) of elements or element ions. That tag is customarily synthesised by adding the appropriate element salts in the form of a solution to the beads. In general, therefore, the elements or element salts are not covalently bonded. Surprisingly, sufficient quantities of those elements or element ions nevertheless remain bound to the bead during the subsequent reaction steps and can be clearly identified at the end of the synthesis of the ligand.

A further possible method of applying the tag is by the use of ion beams, to which the beads are exposed.

In order to achieve more accurate identification, it is customary to use element ions having an atomic mass of more than 80 and preferably an atomic mass of more than 100. In addition, care has to be taken to use pure starting materials and to take account of any impurities formed, for example, as a result of the detection method (e.g. tungsten ions formed by heating the beads using a tungsten coil) or added in the course of the reactions for the synthesis of the ligand. If the use of a specific group of elements is desired, that can, where necessary, be made possible, for example, by a suitable change to the configuration of the equipment for the identification or by the use of different reaction steps in the synthesis of the ligand.

In order to increase the number of codable building blocks it is possible, in addition, to use different isotopes of an element. It is also conceivable, in order further to increase the sensitivity, to start from radioactive isotopes, which can then be identified directly by the nature/energy of their radiation. Since the identification of radioactive isotopes is not dependent on mass, there is no preference as described above for an atomic mass of more than 80.

A further possible method comprises clearly coding each unit of the ligand several times. For example, three different elements or element salts can be used to code a synthesis step, although coding using a single element or element salt would be sufficiently clear (redundant coding). The result is an increase in the reliability of identification, since unclear coding by one element or element salt can be double-checked using the two additional codings.

The elements or elements salts are customarily used in the form of water-soluble salts or complexes. Preference is given to element salts containing Rb, Sr, Y, Zr, Ru, Rh, Pd, Ag,



Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Os, Ir, Pt, Tl, Pb, Bi, Th, W or U. Preference is given especially to salts, such as nitrates, hydroxides or chlorides.

There are to be understood by the expression "solid or semisolid carriers" macroscopic particles which are insoluble in the reaction media and to which sufficient amounts of both the ligand and the tag can be bound. The amount of tag that can be bound to a carrier must be large enough to produce a clearly identifiable signal during analysis of the tag (e.g. > 0.1 fmol or preferably > 1 fmol).

The ligand is bound to the surface of the carrier *via* reactive groups, such as amino, carboxy, hydroxy or halogen groups. Those reactive groups are usually already part of the carrier, but they can be applied or modified subsequently. The resins that are customary in solid phase synthesis, such as those used in Merrifield peptide synthesis, can be used. Those resins generally consist of a basic polystyrene structure partially crosslinked by copolymerisation with divinyl benzene. In order to attach the reactants in solid phase synthesis the basic structures are additionally derivatised. Those modified beads thus have, for example, polyethylene glycols or polyamides on their surfaces.

The binding of the tag to the bead has not been explained, but is possibly the result of the formation of complexes or of inclusions or steric effects. For those reasons, preference is given to resins having groups that are capable of complex formation, such as free amino, ether or hydroxyl groups. In addition, in a preferred form the beads have a diameter of from 0.1 to 0.8 mm.

A further subject of the invention is a process for the production of the library described above, which process comprises adding, during the synthesis of a ligand, before, after or during a synthesis step that is to be coded, a combination of elements or element salts that codes for that synthesis step. Since special reaction steps to bind the element or element ions permanently are not required, the elements or element ions are probably non-covalently bonded to the bead. Preference is given to the use of water-soluble element salts for such reactions. It is customary to use nitrates, hydroxides or chlorides. The use of the elements or element salts in the form of complexes may also be possible.

The process for the production of that coded library comprises, for example:  
a) optionally binding a linker for the ligand to the solid or semisolid carrier;

- b) optionally binding non-variable building blocks of the ligand;
- c) dividing the solid or semisolid carriers into portions for the variable building blocks of the ligand;
- d) in each portion separately, making further modifications to the ligand or attaching another of the variable building blocks that are possible for this position of the ligand and before, after or during the synthesis of the ligand adding a combination of element salts that codes for each synthesis step;
- e) mixing the portions together;
- f) optionally binding non-variable building blocks of the ligand or carrying out reactions that are not to be coded;
- g) repeating steps b) to f) until the variable portion of the ligand has been completely synthesised; and
- h) optionally binding further non-variable building blocks to the ligand.

The execution of steps a), b), f) and h) is dependent on whether the ligand contains constituents that remain constant over the entire library and therefore do not need to be identified by coding. One or more of those constant regions can occur, for example, at the beginning or at the end of the synthesised ligands or within the synthesised ligands.

A preferred method of applying the element salts is, for example, as follows: after the addition of the element salts, the pH value of the solution is adjusted briefly to acid and then to neutral or basic. That allows insoluble element hydroxides that are formed at neutral or basic pH values to be precipitated directly at the bead. The incubation with the element salts customarily lasts from 5 to 50 minutes. The precise incubation period depends upon the salt used, upon the solid or semisolid carrier and upon the ligand already synthesised and therefore in individual cases may also lie outside that range.

The attachment of the first building block of the ligand or of the linker for the first building block is dependent upon the nature of the reactive groups selected and is carried out in accordance with the processes customary for those groups.

The further synthesis is carried out in accordance with generally known methods of solid phase synthesis (Fields *et al.*, Intern. J. Pept. Prot. Res. (1990), 35, 161-214). For the synthesis of the ligand it is customary to use transient protecting groups (terminal protecting groups which are removed before each synthesis step).

Examples of the combination of types of protecting groups are generally known, such as the combination of the Fmoc, Trt, allyloxycarbonyl, Boc, fluorenyl-methyl, nitrophenylsulfenyl, nitrobenzyl, nitroveratryloxycarbonyl and NPS types. A general description of the groups that can be used is to be found, for example, in Fields *et al.* (Intern. J. Pept. Prot. Res. (1990), 35, 161-214).

The reaction steps that are required, for example, for the synthesis of amide bonds are generally known in the art and generally depend upon the way in which the carboxylic acid group participating in the reaction is activated. The reactions generally proceed in the presence of a condensation agent or, when the carboxylic acids are activated in the form of anhydrides, in the presence of an agent that binds the carboxylic acid that is formed. If necessary, chaotropic reagents, such as LiF in N-methylpyrrolidone, can also be added. The reactions are carried out at temperatures of from -30°C to +150°C, preferably at from +10°C to +70°C and especially at from +20°C to +50°C, and can, where appropriate, also be carried out under an inert gas.

Examples of customary condensation agents are carbodiimides, such as N,N'-diethyl-, N,N'-diisopropyl-, N,N'-dicyclohexyl- or N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide; carbonyl compounds, such as carbonyldiimidazole; 1,2-oxazolium compounds, such as 2-ethyl-5-phenyl-1,2-oxazolium-3'-sulfonate and 2-tert-butyl-5-methylisoxazolium perchlorate; acyl-amino compounds, such as 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; and uronium compounds, such as 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU); or phosphonium compounds, such as benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yl-oxy-pyrrolidino-phosphonium hexafluorophosphate (PyBOP).

Customary acid-binding agents are, for example, alkali metals, carbonates or hydrogen carbonates, such as sodium or potassium carbonate or hydrogen carbonate (customarily together with a sulfate), or organic bases, such as sterically hindered tri-lower alkylamines, such as N,N-diisopropyl-N-ethylamine.

Reactive side chains of the building blocks of the ligand that are not intended to participate in the reactions can be protected, for example, by another group of protecting groups. Protecting groups that can be used and procedures for their introduction and removal are described, for example, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973; "Methoden der organischen Chemie", Houben-Weyl, 4th edition,

Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974; Th. W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981; Atherton *et al.*, "Solid phase peptide synthesis - A practical approach" IRL Press Oxford University, 1984; Jones, "The chemical synthesis of peptides", Oxford Science Publications, Clarendon Press Oxford, 1991; and Bodanszky, "Peptide Chemistry", Springer Verlag Berlin, 1988.

Examples of hydroxy-protecting groups are acyl radicals, such as the tert-butoxycarbonyl radical, etherifying groups, such as the tert-butyl group, and silyl and tin radicals, such as the tri-n-butyltin radical or tert-butyldimethylsilyl.

Carboxy groups are protected, for example, by ester formation with groups of the tert-butyl type, benzyl, trimethylsilylethyl or 2-triphenylsilyl groups.

For the protection of amino groups there are used, for example, readily removable acyl-amino, arylmethylamino, esterified mercaptoamino, 2-acylalk-1-enylamino, silylamino, tin-amino or azide groups, such as tert-butoxycarbonyl, allyloxycarbonyl, benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, diphenylmethoxycarbonyl, nitrophenylsulfenyl, 2,2,2-trichloroethoxycarbonyl, pentamethylchromansulfonyl (PMC) or methoxytrimethylbenzylsulfonyl (Mtr) protecting groups.

Thiols are protected, for example, by acetamidomethyl groups.

Those protecting groups are customarily removed together, once the synthesis of ligand and tag is complete, in accordance with methods that are customary in peptide chemistry, such as by treatment with 95 % trifluoroacetic acid. Where appropriate, it is also possible to add strong nucleophilic compounds, such as 1,2-ethane-dithiol, to capture the protecting groups as they are freed.

Further methods of removing those protecting groups are generally known and are based, for example, on  $\beta$ -elimination, solvolysis, hydrolysis, alcoholysis, acidolysis or treatment with a base, or are effected by reduction.

The number of portions for the reactions described under d) (see above) preferably corresponds in each case to the number of possibilities for the next building block of the ligand that is to be attached.

In order to identify the ligand that binds to the desired acceptor, the acceptor to be investigated is added to the library created above, and the carriers to which the acceptor remains bound are washed and identified.

Possible acceptors within the context of the invention are macromolecular units that exhibit a binding affinity for one or more ligands. Examples are receptors, such as serotonin receptors, ROR/RZR receptors, GABA receptors and benzodiazepine receptors; transport proteins, such as Na and K channels, antibodies, transcription activators and enzymes, such as proteases, thrombin, renin, ACE, aromatase and reverse transcriptase; or fragments thereof. The ligands that bind to those acceptors act, for example, as antagonists, inhibitors or agonists, or serve as labels for the acceptors.

In addition, the acceptors are customarily provided with an identifiable group, such as a fluorescent, chemoluminescent or radioactive group, avidin, biotin, a reporter enzyme, an immunologically detectable group (ELISA) or the like. Methods of labelling using the above-mentioned labels and affinity chromatography methods are generally known in the art.

The carriers to which an acceptor is bound are isolated, for example, manually, by sorting the carriers, for example under UV or blue light in the case of fluorescent acceptors, or automatically, using equipment such as that used for the sorting of cells.

In order to exclude false-positive results from the binding test, the ligands can be separated from the isolated carriers and subjected, in homogeneous solution, to a further test, for example a repeat of the test for their binding to the acceptor. The ligand is separated, for example, by cleavage at the linker between the carrier and the ligand using a reaction specific to that linker. When p-hydroxymethylbenzoic acid is used as linker that cleavage can be achieved, for example, by treating the isolated carriers with gaseous ammonia or with a saturated ammonia/THF solution (for example in a microtitre plate). The ligand thus separated can then be removed from the carrier by rinsing and tested again for its binding to the acceptor, or a further test can be carried out that cannot be carried out with the ligands bound to the carriers, such as binding studies or tests in which light absorption is measured, or MS, IR or NMR analyses.

The beads thus identified are customarily isolated and the composition of the ligands bound thereto is identified by the presence of certain elements or element ions.

There may be used for the identification methods that are customarily used in the analysis of elements or element ions, such as total reflection X-ray fluorescence spectrometry (TRXF), neutron activation followed by gamma spectrometry, or mass spectrometry. Preference is given especially to the use of inductively coupled plasma-mass spectrometry (ICP-MS; Houk & Thompson, *Mass Spectrometry Reviews* (1988), 7, 425-461; Date, *Spectrochimica Acta Rev.* (1991), 14, 3-32).

There may be mentioned by way of example the synthesis of a library of potential thrombin inhibitors that contain non-natural amino acids and other building blocks and therefore have, for example, enhanced protease resistance.

The library consists, for example, of pentamers having 2 constant and 3 variable positions and having the following composition:

**X - Y - Z - Pro - GABA**

X= 2-cyanobenzenesulfonyl, D-Phe, N-benzylglycine,  $\beta$ -Ala or acetyl

Y= L-Pro, D-Pro,  $\beta$ -Ala or L-Asp

Z= L-Arg, D-Arg,  $\beta$ -Ala, L-Asp or sarcosyl

That composition gives  $5 \times 4 \times 5 = 100$  possibilities for the composition of the ligands. The individual building blocks are coded by the presence or absence of element salts. After screening of that library it is found that the pentamers D-Phe-D-Pro-Arg-Pro-GABA and D-Phe-Pro-D-Arg-Pro-Gaba-amide are effective as thrombin inhibitors.

The invention is explained in more detail below by means of Examples.

Abbreviations used

DCCI	= dicyclohexylcarbodiimide
DCE	= dichloroethane
DCM	= dichloromethane
DICD	= diisopropylcarbodiimide
DIPEA	= diisopropylethylamine
DMA	= dimethylacetamide
DMF	= dimethylformamide
DMAP	= dimethylaminopyridine
DMSO	= dimethyl sulfoxide
Fmoc	= fluorenylmethyloxycarbonyl

GABA	= $\gamma$ -aminobutyric acid
HOBT	= hydroxybenzotriazole
NEt <sub>3</sub>	= triethylamine
OSu	= O-succinimide
Pip	= piperidine
PMC	= 2,2,5,7,8-pentamethylchroman-6-sulfonyl
RT	= room temperature
TFA	= trifluoroacetic acid
Trt	= trityl

Repetitive treatments are carried out by the batch method in accordance with the procedures of solid phase technology that are generally customary in peptide chemistry (Fields *et al.*, Intern. J. Pept. Prot. Res. (1990), 35, 161-214), for example by the addition of reagent solutions to the resin and subsequent filtration (removal of the filtrate by suction under a low vacuum).

#### Example 1

Described is the production of a library consisting of pentamers having 2 constant and 3 variable positions and having the following composition:

#### **X - Y - Z - Pro - GABA**

- X = 2-cyanobenzenesulfonyl, D-Phe, N-benzylglycine,  $\beta$ -Ala, acetyl  
Y = L-Pro, D-Pro,  $\beta$ -Ala, L-Asp  
Z = L-Arg, D-Arg,  $\beta$ -Ala, L-Asp, sarcosyl

The individual building blocks described above are coded by the following dipeptides in the identification structure (see Table 1):

Table 1

Position	Building block	Code
X	2-cyanobenzenesulfonyl chloride D-Phe N-benzylglycine $\beta$ -Ala acetyl	no element Pr La Sb + La Sb
Y	L-Pro D-Pro $\beta$ -Ala L-Asp	no element Nd Eu Nd + Eu
Z	L-Asp D-Arg $\beta$ -Ala L-Arg sarcosyl	no element Tb Ho Tb + Ho Lu

in each case the nitrates of the above metal ions are used

#### Example 1.1: Coupling of the linker for the ligand to the resin

For preactivation, 769  $\mu$ mol of 4-hydroxymethylbenzoic acid are dissolved in 845  $\mu$ mol (based on HOBT) of a 0.5M HOBT/DMA solution and 845  $\mu$ mol (based on DICD) of a 2M DICD/DMA solution are added. After 40 minutes at room temperature, the reaction mixture is diluted with 0.3 ml of DMA and pipetted into the resin. Immediately afterwards, 845  $\mu$ mol of DIPEA are added. The coupling reaction is continued for 60 minutes at room temperature and then rinsing is carried out as follows:

10x for 45 seconds with DMA

5x for 60 seconds with isopropanol

6x for 45 seconds with DMA

Resin A is formed.

#### Example 1.2: Esterification of the C-terminal ligand component with the linker

Batch:

There is pipetted into 154  $\mu$ mol of the modified resin A from Example 1.1 a solution of

615  $\mu$ mol Fmoc-GABA-OH

600  $\mu$ l DMA and

3 ml dichloroethane

To that suspension a solution of:

646  $\mu$ mol DCCI and

307  $\mu$ l dichloroethane



is added in 2 portions over a period of 5 minutes at room temperature and, finally, a solution of:

30.7  $\mu\text{mol}$  DMAP and  
65  $\mu\text{l}$  dichloroethane

is added. After a total of 20 minutes, 154  $\mu\text{mol}$  of N-methylmorpholine are added, the reaction is continued for 4 hours and then rinsing is carried out as follows:

4 $\times$  for 45 seconds with DMA  
3 $\times$  for 45 seconds with dichloroethane  
4 $\times$  for 45 seconds with DMA

Resin B is formed.

#### Example 1.3: Chain-lengthening of the constant portion of the ligand

The Fmoc group is removed by treatment with 20% Pip/DMA (15 $\times$  for 0.5 min each time). The combined filtrates are diluted in the manner defined and their extinction is measured at 299.8 nm. A total of approximately 154  $\mu\text{mol}$  of free amino groups is found.

For preactivation, 367  $\mu\text{mol}$  of Fmoc-Pro-OH are dissolved in 404  $\mu\text{mol}$  (based on HOBT) of a 0.5M HOBT/DMA solution and 404  $\mu\text{mol}$  (based on DICD) of a 2M DICD/DMA solution are added thereto. After 40 minutes at room temperature, the reaction mixture is diluted with 1.3 ml of DMA and added to 122  $\mu\text{mol}$  of resin B from Example 1.2. Immediately afterwards, 404  $\mu\text{mol}$  of DIPEA are added and the coupling reaction is continued for 45 minutes at room temperature. A subsequent coupling reaction using the same amount of Fmoc-Pro (as above) follows in order to increase the yield (a further 35 minutes). The resin is then rinsed as follows:

5 $\times$  for 45 seconds with DMA  
5 $\times$  for 45 seconds with isopropanol

Resin C is formed.

Resin C is dried and divided into smaller portions suitable for the incorporation of the variable positions Z. The incorporation of the variables Z in this Example requires 5 portions for the separate reactions with 5 different building blocks ("split synthesis").

#### Example 1.4: Synthesis of the variable portions of the ligands

5 portions (pools) each of 19  $\mu\text{mol}$  are produced (one pool for each of the variables possible at that position).

**Example 1.4.1: Synthesis of the 1st variable position of the ligand**

First, the Fmoc group is removed from the ligand as described in Example 1.3. The separate coupling reactions for the 1st variable position (Z position) are then carried out:

Pool	Building block	Amount	Coding
1	Fmoc-L-Arg(PMC)-OH	76 $\mu$ mol	Tb + Ho
2	Fmoc-D-Arg(PMC)-OH	76 $\mu$ mol	Tb
3	Fmoc- $\beta$ -Ala-OH	76 $\mu$ mol	Ho
4	Fmoc-L-Asp(OtBu)-OH	76 $\mu$ mol	-
5	Fmoc-sarcosyl-OH	76 $\mu$ mol	Lu

In each case one of the Fmoc building blocks listed above is preactivated with 84  $\mu$ mol of HOBT solution (0.5M in DMA) and 84  $\mu$ mol of DICD solution (2M in DMA) for 40 minutes at room temperature and, after dilution with 230  $\mu$ l of DMA, added to a portion of the resin. Immediately afterwards 84  $\mu$ mol of DIPEA are added. Coupling takes place for a period of 45 minutes at room temperature. Post-coupling is carried out for a further 35 minutes with a mixture of the same composition. Then rinsing is carried out as described in Example 1.3.

**Example 1.4.2: Incorporation of the binary tag**

In accordance with the code table the tag is introduced, in each case after the coupling of the Z, Y and X components, by means of an approximately 15 minute period of incubation at room temperature with metal salt solutions (or mixtures, see code table). The concentration of the starting solutions (in the form of nitrate salts) is a standard 1 g of element/litre. The elements are >99.99% pure. According to the code that is to be introduced, equal volumes of the appropriate salt solutions are mixed together. In the case of ligands carrying acid-labile protecting groups (tert-but.), shortly before incubation of the carrier the pH is adjusted from approx. 1 to approx. 4-5 with aqueous NaOH in order to avoid removal of those protecting groups. Slight cloudiness occurs as a result of precipitated metal hydroxides which does not, however, affect the tagging. In the case of ligands that are not sensitive to acids, the reaction can be carried out at pH 1. The individual portions of resin (each approx. 0.1 g), are treated 5x for 0.5 min each time with water and for 15 minutes with 2 ml each of metal salt solution at the above concentration (pH 4-5) and in accordance with the code. The portions are then washed intensively with water 5x for 2 min each time in order to prevent cross-contamination after mixing by the 'split-mix' process.

**Example 1.4.3: Synthesis of the 2nd variable position of the ligand**

Position Y is then synthesised analogously to the incorporation of Z (taking account of the different codings). For that purpose all the samples from Example 1.4.2 are mixed together and divided into 4 portions of equal size (4 Y portions).

In each of those 4 separate portions one of the building blocks possible at that position is attached as described in Example 1.4.1.

Pool	Building block	Amount	Coding
1	Fmoc-L-Pro-OH	76 $\mu$ mol	-
2	Fmoc-D-Pro-OH	76 $\mu$ mol	Nd
3	Fmoc- $\beta$ -Ala-OH	76 $\mu$ mol	Eu
4	Fmoc-L-Asp(OtBu)-OH	76 $\mu$ mol	Nd + Eu

Then, as described in Example 1.6.3, the metal salt coding for that particular building block is applied.

**Example 1.4.4: Synthesis of the 3rd variable position of the ligand**

Position X is synthesised analogously to the incorporation of Y and Z (taking account of the different codings). Then all the samples from Example 1.4.3 are mixed together and divided into 5 portions of equal size (5 X portions).

In each of those 5 separate portions one of the building blocks possible at that position is attached as described in Example 1.4.1.

Pool	Building block	Amount	Coding
1	cyanobenzenesulfonyl chloride	76 $\mu$ mol	-
2	Fmoc-D-Phe-OH	76 $\mu$ mol	Pr
3	Fmoc-N-benzylglycine-OH	76 $\mu$ mol	La
4	Fmoc- $\beta$ -Ala-OH	76 $\mu$ mol	Sb + La
5	acetic anhydride	76 $\mu$ mol	Sb

The coupling of the building block 2-cyanobenzenesulfonyl chloride is effected by simple reaction with a solution of the sulfonyl chloride in pyridine/DMA at room temperature, and the coupling reaction for the acetic anhydride is effected as described in Example 1.5.

Then, as described in Example 1.4.2 or 1.4.3, the metal salt coding for the particular building block is applied.

#### Example 1.5: Deprotecting the ligand

An appropriate number of resin particles from the library present in protected form (but a multiple of the number of components) is subjected to the deprotection reactions for the removal of protecting groups of the PMC and tert-butyl type customary in peptide chemistry. In order to remove the PMC protecting groups, the resin from Example 1.4.4 is treated with 95% trifluoroacetic acid (5% water as scavenger + 2% ethanedithiol) for 40 minutes at room temperature. In order to remove the Fmoc protecting groups, the resin is treated repeatedly with 20% Pip/DMA at room temperature (see Example 1.2). Rinsing is then carried out as follows:

- 4× for 45 seconds with DCE
- 4× for 45 seconds with isopropanol
- 4× for 45 seconds with isopropanol:H<sub>2</sub>O = 1:1 (v:v)
- 5× for 45 seconds with H<sub>2</sub>O.

#### Example 1.6: Labelling of thrombin with fluorescein

Fluorescein isothiocyanate (10 µl of a solution of 2.9 mg in 290 µl of DMSO) is added to a solution of human thrombin (0.53 mg in 100 µl of borate buffer pH 8). The reaction is continued for 60 minutes at room temperature and the reaction mixture is worked up on a gel-chromatography column (Sephadex<sup>®</sup>-G25, 15×0.5 cm) (elution with 1M NaCl).

20 µl of the fluorescein/thrombin fraction (approx. 3% of the fraction volume) are diluted to 500 µl and analysed photometrically at 495 nm. A fluorescein concentration of approximately 2 µM is obtained. The fluorescein/thrombin ratio is thus approx. 1:1.

#### Example 1.7: Identification of thrombin-binding ligands in heterogeneous phase

Approx. 500 beads of the resin from Example 1.5 are incubated for about 10 minutes at room temperature in a 2.2 µM solution of fluorescein-labelled thrombin from Example 1.6. After rinsing 3× for 1 minute each time with a solution of:

68 mg imidazole  
876 mg NaCl  
147 mg  $\text{CaCl}_2 \times 2\text{H}_2\text{O}$   
3.35 g PEG (3350)  
ad. 100 ml  $\text{H}_2\text{O}$ ,

the beads are examined visually for inherent fluorescence under long-wavelength light (blue light at approx. 470 nm). Particles that are clearly fluorescent are isolated and rinsed

5× for 3 minutes with formamide and

10× with  $\text{H}_2\text{O}$

and then dried.

Example 1.8: Testing the inhibition of thrombin by ligands from individual beads in liquid phase

The particles isolated in Example 1.7 are exposed in the dry state to an ammonia gas atmosphere (approx. 1 bar) for 24 hours. They are then distributed individually into numbered wells in a microtitre plate having filter plates (e.g. the Millipore MultiScreen DV96 model, pore size 0.65  $\mu\text{m}$ ). Using a tetrahydrofuran/water mixture, the ligands can be flushed into a second numbered microtitre plate where they are tested directly in a thrombin inhibition assay. There are added per well  $2.3 \times 10^{-2}$  NIH units of thrombin and 9.3  $\mu\text{g}$  of chromogenic substrate S-2302 (D-Pro-Phe-Arg-p-nitroanilide 2HCl; Chromogenix) in a final volume of 150  $\mu\text{l}$ . The colour development is measured over a period of approx. 75 min using a Multiwell-Platereader at a wavelength of 405 nm and the corresponding extinction values are shown as a function of time. Cross-comparisons of the gradients of curves for different ligands serve as a basis for the identification of ligands having inhibitor activity. The corresponding beads can then be identified.

Example 1.9: Analytical determination of the element composition in individual particles by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry)

The elements used for coding are identified by inorganic mass spectrometry (Inductively Coupled Plasma-Mass Spectrometry - ICP-MS) (Houk & Thompson, *Mass Spectrometry Reviews* (1988), 7, 425-461; Date, *Spectrochimica Acta Rev.* (1991), 14, 3-32). A VG PlasmaQuad (Fisons Instruments, Winsford, UK) is used. The supply of samples to that apparatus has been modified; instead of a pneumatic atomiser, the apparatus is equipped with a device for electrothermal vaporisation (ETV) of the sample (Barth & Krivan, *Journal of Analytical Atomic Spectrometry* (1994), 9, 773-777). The individual bead is applied to a

tungsten coil and atomised on that coil at 2500 - 2700°C. The atom cloud is conveyed into the plasma by the flow of carrier gas (Ar/H<sub>2</sub>). The sample is ionised in the argon plasma at 6000 - 8000°C. The elements are separated by mass spectrometry using a quadrupole. The signals are detected by a Channel Electron Multiplier and processed in scanning mode by a Multichannel Analyzer. This application is concerned exclusively with the qualitative identification of the elements. For reasons specific to the method, elements having atomic masses of <80 amu are excluded from the coding since at low masses the spectral background is distorted by a large amount of interference. In addition to the element tungsten, other elements present as contaminants in the tungsten coil are not taken into consideration for the coding.

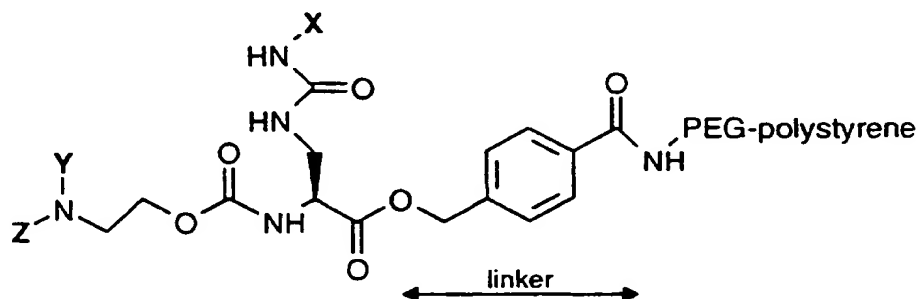
The detection limit for the individual elements is estimated to be approx. 1 - 10 pg. Assuming an atomic mass of 100, that corresponds to from 10<sup>-14</sup> to 10<sup>-13</sup> mol.

Some of the best beads contain the elements Pr, Nd, Tb and Ho. According to the code table, those elements code for D-Phe-D-Pro-Arg, i.e. for the ligand D-Phe-D-Pro-Arg-Pro-Gaba-amide.

Another set of good beads contains the elements Pr and Tb. That combination codes for the ligand D-Phe-Pro-D-Arg-Pro-Gaba-amide.

### Example 2:

A library based on aminopropionic acid (Dpr) as template is produced.



The building blocks for the variables Y and Z are coded with triple redundancy (see Table 2).

The library consists of 3968 different compounds wherein

X= 775-780, 70, 71, 72, 73, 74, 75, 781-790, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 791-800, 86, 87, 88, 89, 90, 91, 92, 94, 95, 97, 801-810, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 811-820, 111, 112, 113, 115, 116, 117, 118, 119, 120, 131, 821-830, 135, 143, 144, 145, 146, 147, 148, 149, 150, 151, 831-836, 552, 553, 554, 555, 556, 557;

Y= 504, 507, 511, 512, 515, 527, 536, 538;

Z= 70, 71, 76, 85, 100, 104, 109, 111

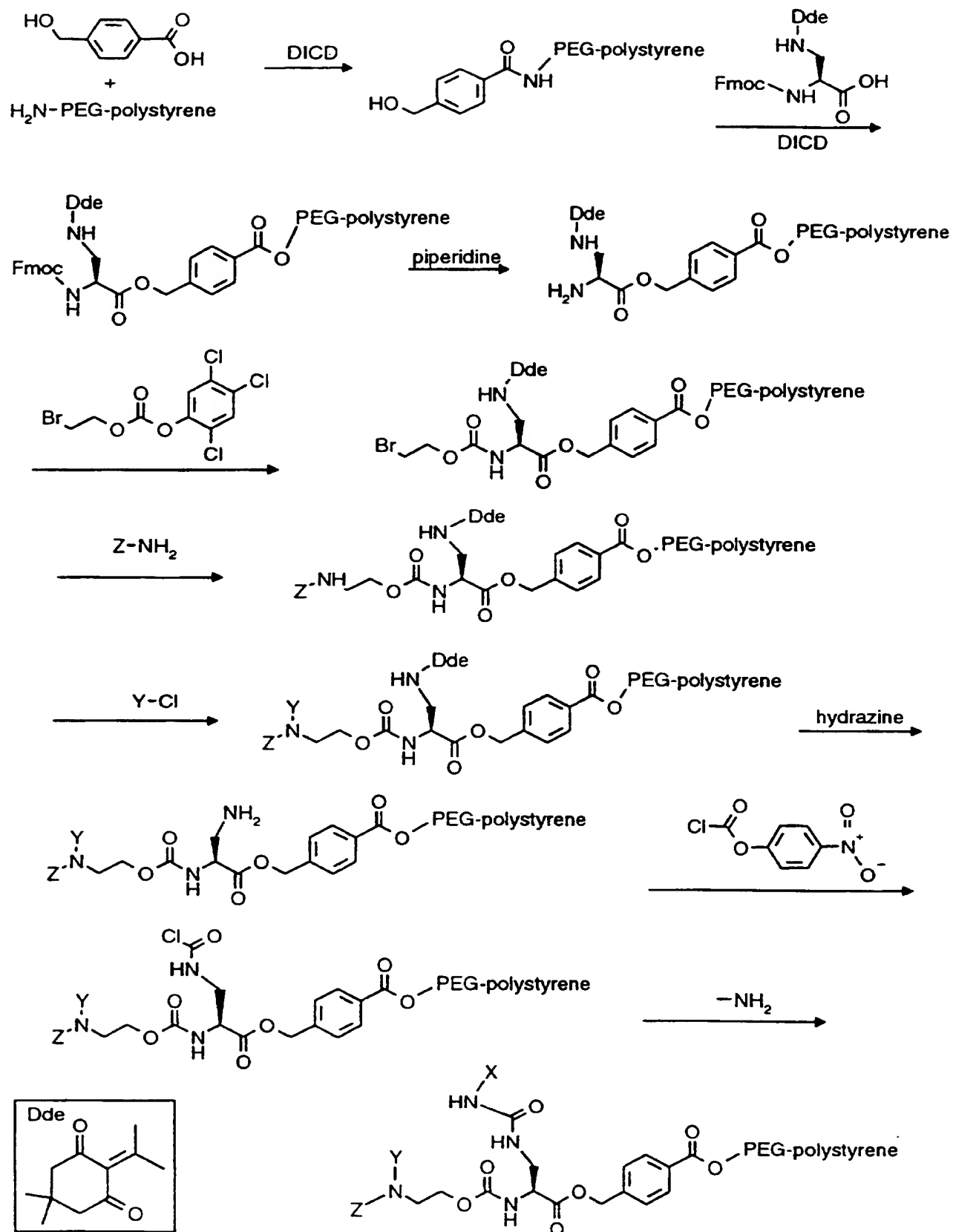
The building blocks used are shown in Table 4

Example 2.1: Coding of the building blocks

Table 2

			Ba	Bi	Cd	Ce	Cs	Dy	Er	Eu	Gd	Hf	Ho	In	Ir	La	Lu	Nd	Os	Pb
bb																				
			Z: 3 elements/bit																	
70			0	0	0	0	0	0	0	0	0									
71			0	0	1	0	0	1	0	0	1									
76			0	1	0	0	1	0	0	1	0									
85			0	1	1	0	1	1	0	1	1									
100			1	0	0	1	0	0	1	0	0									
104			1	0	1	1	0	1	1	0	1									
109			1	1	0	1	1	0	1	1	0									
111			1	1	1	1	1	1	1	1	1									
															Y: 3 elements/bit					
504												0	0	0	0	0	0	0	0	0
507												0	0	1	0	0	1	0	0	1
511												0	1	0	0	1	0	0	1	0
512												0	1	1	0	1	1	0	1	1
515												1	0	0	1	0	0	1	0	0
527												1	0	1	1	0	1	1	0	1
536												1	1	0	1	1	0	1	1	0
538												1	1	1	1	1	1	1	1	1
			Ba	Bi	Cd	Ce	Cs	Dy	Er	Eu	Gd	Hf	Ho	In	Ir	La	Lu	Nd	Os	Pb

bb: building block (see Table 4)





**Example 2.2: Coupling of the linker 4-hydroxymethylbenzoic acid to amino-PEG-polystyrene**  
**(Tentagel S Novabiochem, 130  $\mu$ m diameter)**

5.4 mmol (amino group) = 18.6 g of carrier are washed with approximately 50 ml of DMA, 50 ml of 20% piperidine in DMA and 6x with 50 ml of DMA each time. 16.2 mmol (2.46 g) of 4-hydroxymethylbenzoic acid are preactivated in 17.8 mmol (35.6 ml; 0.5M in DMA) of HOBt and 17.8 mmol (8.9 ml; 2M in DMA) of DICD for 40 minutes at room temperature and then added to the carrier and mixed. After 2 minutes, 16.2 mmol (2.8 ml) of DIPEA are added and mixed in, and the reaction mixture is maintained at room temperature for 60 minutes. The reaction solution is filtered with suction and washed 8x with DMA, 4x with isopropanol and 4x with THF (50 ml each time). The resin is dried *in vacuo*.

**Example 2.3: Coupling of Fmoc-Dpr(Dde)**

A mixture of 2.32 mmol (1.14 g) of Fmoc-Dpr(Dde)-OH in 2.55 mmol (5.1 ml; 0.5M in DMA) of HOBt and 2.55 mmol (1.28 ml; 2M in DMA) of DICD and, after two minutes, 2.32 mmol (397  $\mu$ l) of DIPEA are added to 580  $\mu$ mol (approx. 2.0 g) of the above carrier. After 2 hours at room temperature, the reaction mixture is washed 3x with DMA and unreacted OH groups are acetylated over a period of 5 minutes with 20 ml of a mixture of acetic anhydride/pyridine/DMA (1:1:8 vol). Washing is carried out 5x with DMA and 5x with isopropanol (20 ml each).

**Example 2.4: Removal of Fmoc**

The resin is maintained at room temperature for 17 minutes with approx. 30 ml of 20% piperidine in DMA and then washed 5x with DMA and 5x with isopropanol and 5x with DMSO (20 ml each).

**Example 2.5: Acylation with bromoethoxy-carbonyl-2,4,5-trichlorophenyl ester**

The resin is maintained at room temperature for 2 hours with 3.45 mmol (1.20 g) of bromoethoxy-carbonyl-2,4,5-trichlorophenyl ester in 3.5 ml of DMA and 345  $\mu$ mol (230  $\mu$ l; 1.5M in DMA) and washed 6x with DMSO and 4x with THF (20 ml each).

**Example 2.6: Reaction with Z-amines and tagging of Z**

The resin is divided into 8 portions and reacted for 2 hours at room temperature with the amines 70, 71, 76, 85, 100, 104, 109, 111 (see Table 4; 1.72 mmol of each) in 0.7 ml of

DMSO. The resin is then washed 3x with DMSO, 6x with DMA and 3x with water (approx. 5 ml each).

Coding (tagging): 0.75 ml each of salt solutions containing the elements (0.1%) corresponding to Table 2 in water is adjusted to approx. pH 5 with 2N NaOH and the resin portions are treated for 30 minutes at room temperature. The resin portions are washed 3x with water, 2x with DMA, 4x with water (5 ml each) and then combined and washed a further 8x with DMA (20 ml).

Example 2.7: Acylation with Y-sulfonyl chlorides

The resin is divided into 8 portions and reacted for 2 hours at room temperature with 215  $\mu$ mol of the sulfonyl chlorides 504, 507, 511, 512, 515, 527, 536, 538 (see Table 4) in 1.0 ml of DMA/pyridine (1:1 vol) containing 215  $\mu$ mol (37  $\mu$ l) of DIPEA. The resins are washed 6x with DMA, 3x with isopropanol and 6x with water (approx. 5 ml each).

Coding: 0.75 ml each of salt solutions containing the elements (0.1%) corresponding to Table 2 in water is adjusted to approx. pH 5 with 2N NaOH and the resin portions are treated for 30 minutes at room temperature. The resin portions are washed 3x with water, 2x with DMA, 4x with water (5 ml each) and then combined and washed a further 8x with DMA (20 ml).

Example 2.8: Removal of Dde

The resin is reacted for 6 minutes at room temperature with 20 ml of 2 % hydrazine hydrate in DMA and washed 6x with DMA and 4x with THF/DCM (1:1 vol) (approx. 20 ml each).

Example 2.9: Acylation with chloroformic acid p-nitrophenyl ester

The resin is reacted for 30 minutes at room temperature with 8.6 mmol (1.74 g) of chloroformic acid p-nitrophenyl ester and 8.6 mmol (1.48 ml) of DIPEA in 17.2 ml of THF/DCM (1:1 vol). The resin is washed 6x with THF/DCM (1:1 vol).

Example 2.10: Reaction with X-amines

The resin is divided into 62 portions and reacted for 2.5 hours at room temperature with 140  $\mu$ mol of the following amines: 70 - 92, 94, 95, 97, 99 - 108, 111, 112, 113, 115 - 120, 131, 135, 143 - 151, 552 - 557 (see Table 4), in each case together with 140  $\mu$ mol of DIPEA. The resins are washed 4x with DMA, 4x with isopropanol and 4x with DCM (approx.

1 ml each). Since the resin portions (=sublibraries) are no longer mixed, X does not need to be coded with a tag.

**Example 2.11: Removal of tert-butyl protecting groups (e.g. in building block 89)**

The resin portions are each maintained at room temperature for 20 minutes with 0.5 ml of trifluoroacetic acid/water/DCM (48:2:50 vol) and then washed as follows: 3x with DCM, 3x with THF, 2x with DMA, 2x with isopropanol, 2x with DMA and 3x with isopropanol (approx. 1 ml each). The resins are dried *in vacuo*.

That library can be used for assays on the particles. However the ligands can also be cleaved using nucleophilic compounds (e.g. morpholine, propylamine, ammonia, etc.) and tested in solution. Positive ligands can then be found by means of tag analysis:

e.g. a positive bead from the library wherein X = building block 73 contains the elements Bi, Cd, Cs, Dy, Eu, Gd and Ho, In, La, Lu, Os, Pb:

decoding gives: Z = building block 85 and Y = building block 512.

Removal of the ligand using n-propylamine (RT, 24 h) yields a material that according to ES-MS has a mass of  $M^+H^+ = 848$  (M expected = 846.99)

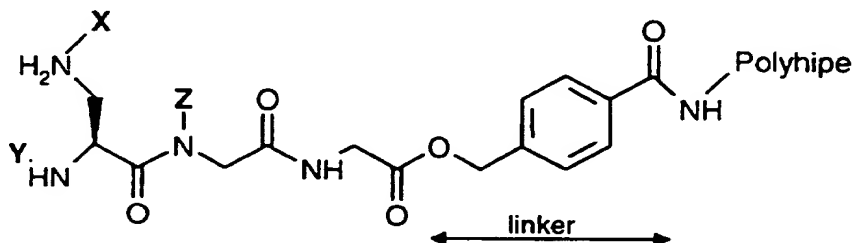
e.g. a positive bead from the library wherein X = building block 115 contains the elements Ba, Bi, Ce, Cs, Er, Eu and Hf, Ho, In, Ir, La, Lu, Nd, Os, Pd:

decoding gives  $Z = \text{building block 109}$  and  $Y = \text{building block 538}$

Removal of the ligand using n-propylamine (RT, 24 h) yields a material that according to ES-MS has a mass of  $M^+H^+ = 646$  (M expected = 644.80).

### Example 3:

A library based on the following template is produced



The building blocks for the variables Y and Z are coded with triple redundancy (see Table 3).

- 26 -

X= 1 - 20, 51, 53 - 56, 58, 60, 63, 66, 69, 319 - 323, 325, 327 - 332, 334, 335, 336, 338,  
339, 340, 341, 344, 392, 398, 399, 401, 402, 413, 502, 503, 505 - 510, 512, 514, 515,  
517, 518, 519, 521, 523 - 551

Y= 505, 507, 512, 514, 515, 517, 518, 519, 521, 525

Z= 70, 71, 72, 74, 79, 80, 84, 85, 97, 102

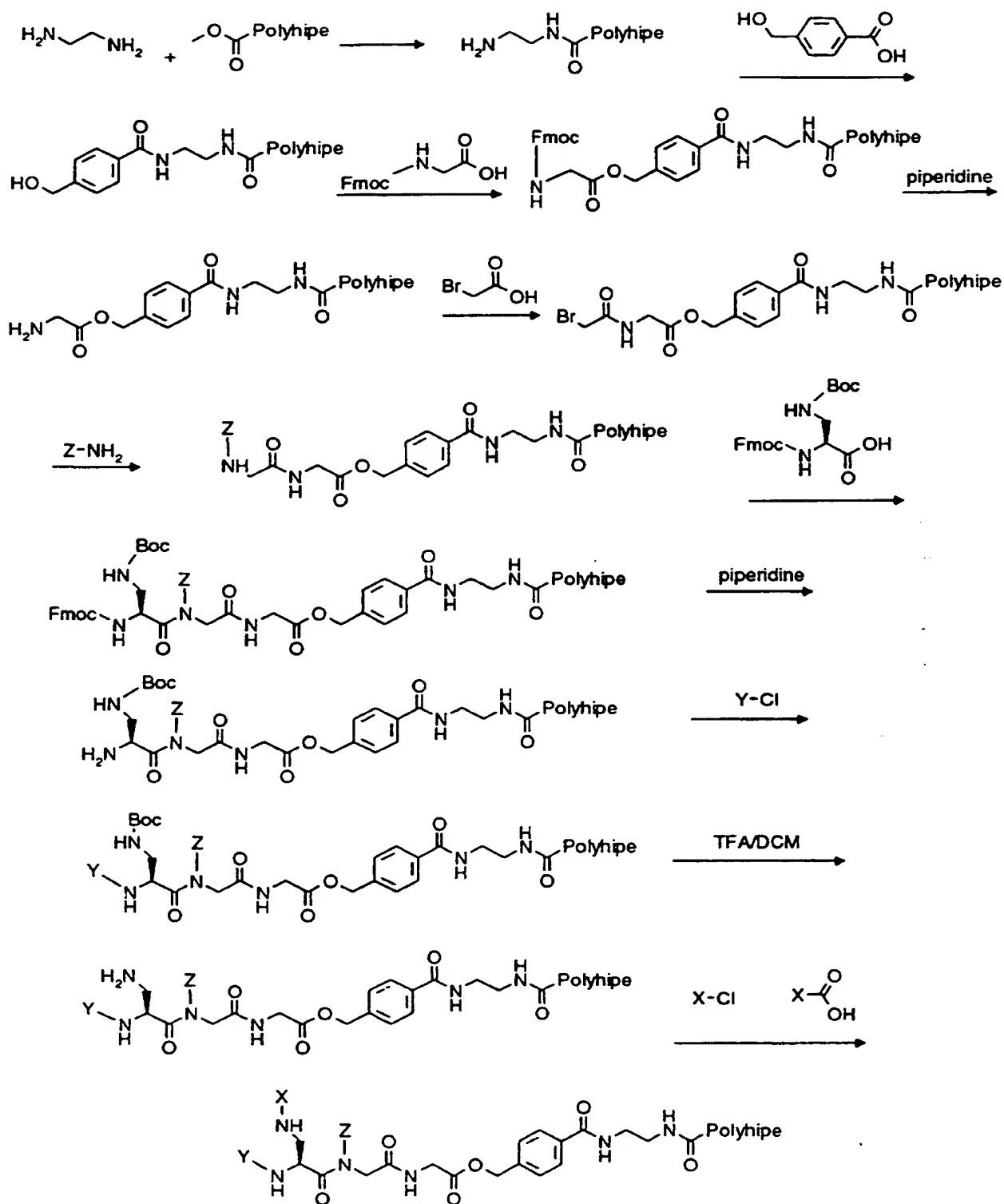
The building blocks used are shown in Table 4.

### Example 3.1: Coding of the building blocks

Table 3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Bi	Eu	Ho	Ir	Lu	Os	Tb	Te	Ag	La	Nd	Pd	Pr	Sb	Tl	Yb	Ba	Ce	Cs	Dy	Gd	Hf	Rh	Y	Cd
bb																									
	Z: 4 bits / 3 elements/bit																								
70	0	0	0	1	0	0	0	1	0	0	0	1													
71	0	0	1	0	0	0	1	0	0	0	1	0													
72	0	0	1	1	0	0	1	1	0	0	1	1													
74	0	1	0	0	0	1	0	0	0	1	0	0													
79	0	1	0	1	0	1	0	1	0	1	0	1													
80	0	1	1	0	0	1	1	0	0	1	1	0													
84	0	1	1	1	0	1	1	1	0	1	1	1													
85	1	0	0	0	1	0	0	0	1	0	0	0													
97	1	0	0	1	1	0	0	1	1	0	0	1													
102	1	0	1	0	1	0	1	0	1	0	1	0													
	Y: 4 bits / 3 elements/bit																								
505													0	0	0	1	0	0	0	1	0	0	0	1	
507													0	0	1	0	0	0	1	0	0	0	1	0	
512													0	0	1	1	0	0	1	1	0	0	1	1	
514													0	1	0	0	0	1	0	0	0	1	0	0	
515													0	1	0	1	0	1	0	1	0	1	0	1	
517													0	1	1	0	0	1	1	0	0	1	1	0	
518													0	1	1	1	0	1	1	1	0	1	1	1	
519													1	0	0	0	1	0	0	0	1	0	0	0	
521													1	0	0	1	1	0	0	1	1	0	0	1	
525													1	0	1	0	1	0	1	0	1	0	1	0	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Bi	Eu	Ho	Ir	Lu	Os	Tb	Te	Ag	La	Nd	Pd	Pr	Sb	Tl	Yb	Ba	Ce	Cs	Dy	Gd	Hf	Rh	Y	Cd

## Reaction scheme



Example 3.2: Functionalisation of Polyhipe SU 500 (Novabiochem)

The methyl ester functions in the carrier (5 g) are reacted for 20 hours at room temperature with 750 mmol (45.3 g) of diaminoethane and the carrier is washed as follows: 6x with DMA, 6x with isopropanol, 6x with DMA and 6x with isopropanol (approx. 50 ml each).

Example 3.3: Coupling of 4-hydroxymethylbenzoic acid to the carriers obtained above

2.6 mmol (amino group) = approx. 5.5 g of carrier are washed with approx. 50 ml of DMA, 50 ml of 20% piperidine in DMA and 6x with 50 ml of DMA each time. 13.0 mmol (1.98 g) of 4-hydroxymethylbenzoic acid are preactivated for 40 minutes at room temperature in 14.3 mmol (28.6 ml; 0.5M in DMA) of HOBt and 14.3 mmol (7.2 ml; 2M in DMA) of DICD and then added to the carrier and mixed. After 2 minutes, 13 mmol (2.2 ml) of DIPEA are added and the reaction mixture is maintained at room temperature for 20 hours. The reaction solution is filtered with suction and washed 8x with DMA, 4x with isopropanol and 4x with THF (50 ml each). The resin is dried *in vacuo*.

Example 3.4: Coupling of Fmoc-glycine

A mixture of 10.4 mmol (3.09 g) of Fmoc-Gly-OH in 11.4 mmol (22.8 ml; 0.5M in DMA) of HOBt and 11.4 mmol (5.7 ml; 2M in DMA) of DICD and, after 2 minutes, 10.4 mmol (1.78 ml) of DIPEA are added to 2.6 mmol (approx. 6 g) of the above carriers. After 2 hours at room temperature, the reaction mixture is washed 3x with DMA and unreacted OH groups are acetylated for 5 minutes with 20 ml of a mixture of acetic anhydride/pyridine/DMA (1:1:8 vol). Washing is carried out 5x with DMA and 5x with isopropanol (20 ml each).

Example 3.5: Removal of the Fmoc group

The resin is reacted for 20 minutes at room temperature with 30 ml of 20% piperidine in DMA and the reaction mixture is washed 8x with DMA (approx. 20 ml each).

Example 3.6: Coupling of bromoacetic acid

The resin is reacted for 30 minutes at room temperature with 9.8 mmol (1.36 g) of bromoacetic acid and 10.8 mmol (5.4 ml; 2M in DMA) of DICD. The reaction mixture is filtered with suction and the coupling is repeated. The resin is washed 4x with DMA and 4x with DMSO (approx. 20 ml each).

**Example 3.7: Reaction with Z-amines and tagging of Z**

The resin is divided into 10 portions and reacted for 2 hours at room temperature with 7.8 mmol each of the following amines in 3 ml of DMA: 70, 71, 72, 74, 79, 80, 84, 85, 97, 102. The resin portions are washed as follows: 4x with DMSO, 4x with DMA, 4x with water (6 ml each).

Coding: 4.0 ml each of salt solutions containing the elements (0.1%) corresponding to Table 3 in water are adjusted to approx. pH 5 with 2N NaOH and the resin portions are treated for 30 minutes at room temperature. The resin portions are washed 3x with water, 2x with DMA and 4x with water (6 ml each) and then combined and washed a further 8x with DMA (20 ml).

**Example 3.8: Coupling of Fmoc-Dpr(Boc) to the above resin**

A mixture of 5.88 mmol (2.51 g) of Fmoc-Dpr(Boc)-OH in 6.47 mmol (12.9 ml; 0.5M in DMA) of HOBT and 6.47 mmol (3.25 ml; 2M in DMA) of DICD and, after 2 minutes, 5.88 mmol (1.0 ml) of DIPEA are added to the above carrier. After 1.5 hours at room temperature, the reaction mixture is washed 3x with DMA and unreacted OH groups are acetylated for 5 minutes with 30 ml of a mixture of acetic anhydride/pyridine/DMA (1:1:8 vol). The reaction mixture is washed 5x with DMA and 5x with isopropanol (20 ml each).

**Example 3.9: Removal of Fmoc**

The above resin is maintained at room temperature for 20 minutes with approx. 30 ml of 20% piperidine in DMA and washed 5x with DMA and 5x with isopropanol and 5x with DMA (20 ml each).

**Example 3.10: Acylation with Y-sulfonyl chlorides and tagging of Y**

The resin is divided into 10 portions and reacted for 2.5 hours at room temperature with 377  $\mu$ mol of the sulfonyl chlorides 505, 507, 512, 514, 515, 517, 518, 519, 521, 525 in 3.0 ml of DMA/pyridine (1:1 vol) containing 377  $\mu$ mol (65  $\mu$ l) of DIPEA. The resins are washed 6x with DMA, 3x with isopropanol and 6x with water (approx. 5 ml each).

Coding: 4.0 ml each of salt solutions containing the elements (0.1%) corresponding to Table 3 in water are adjusted to approx. pH 5 with 2N NaOH and the resin portions are treated for 30 minutes at room temperature. The resin portions are washed 3x with water, 2x with DMA

and 4x with water (5 ml each) and then combined and washed a further 8x with DMA (20 ml).

#### Example 3.11: Removal of Boc

The resin is treated for 20 minutes at room temperature with TFA (5% water)/DCM (1:1) in order to remove the Boc group. Washing is carried out as follows: 3x with DCM, 3x with DMA, 3x with isopropanol, 8x with DMA (20 ml each).

#### Example 3.12: Acylation of the amino group

The resin is divided into 100 portions and in 27 portions the X-sulfonyl chlorides 505, 507, 512, 514, 515, 517, 518, 519, 521, 523 - 540 are reacted in accordance with the following method:

35  $\mu$ mol of sulfonyl chloride are reacted for 2.5 hours at room temperature in 0.3 ml of DMA/pyridine (1:1 vol) with 35  $\mu$ mol (6  $\mu$ l) of DIPEA. The resins are washed 6x with DMA, 3x with isopropanol and 6x with DMA (approx. 1 ml each).

The remaining 73 portions are reacted with the carboxylic acids 541 - 551, 51, 53 - 56, 1 - 20, 58, 60, 63, 66, 69, 502, 503, 506, 508, 509, 510, 413, 402, 401, 399, 398, 392, 319 - 323, 325, 327 - 332, 334, 335, 336, 338, 339, 340, 341, 344 in accordance with the following method:

35  $\mu$ mol of acid are preactivated for 30 minutes at room temperature with 35  $\mu$ mol of HOBt and 35  $\mu$ mol of DICD in 0.3 ml of DMA; 35  $\mu$ mol (6  $\mu$ l) of DIPEA are added and the reaction solution is added to the resin portions and reacted for 2 hours at room temperature.

Washing is then carried out as follows:

6x with DMA, 3x with isopropanol, 6x with DCM (1 ml each).

#### Example 3.13: Removal of the But and Fmoc protecting groups

The individual resin portions are reacted for 20 minutes with 0.5 ml of TFA (5% water)/DCM (1:1), washed 6x with isopropanol and then maintained at room temperature for 20 minutes with 1 ml of 20% piperidine in DMA. The resin portions are then washed 6x with DMA and 6x with isopropanol and the resins are dried.

The resin portions are no longer mixed but form the individual sublibraries.



That library is used for assays on the particles. However the ligands can also be cleaved using nucleophilic compounds (e.g. morpholine, propylamine, ammonia, etc.) and tested in solution.

Positive ligands are found by means of tag analysis:

e.g. a positive bead from the library wherein X = building block 514 contains the elements Eu, Ho, Os, Tb, La, Nd and Tl, Cs, Rh:

decoding gives: Z = building block 80 and Y = building block 507

Removal of the ligand using n-propylamine (RT, 24 hours) yields a material that according to ES-MS has a mass of  $M^+H^+ = 713$  (M expected = 711.86)

e.g. a positive bead from the library wherein X = building block 60 contains the elements Bi, Ho, Lu, Tb, Ag, Nd and Sb, Tl, Yb, Ce, Cs, Dy, Hf, Rh, Y:

decoding gives Z = building block 102 and Y = building block 518

Removal of the ligand using n-propylamine (RT, 24 hours) yields a material that according to ES-MS has a mass of  $M^+H^+ = 852$  (M expected = 851.01).

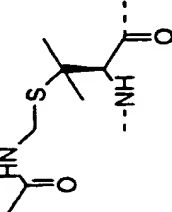
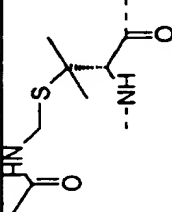
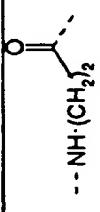
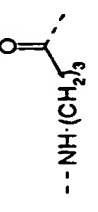
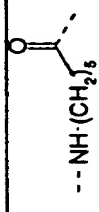

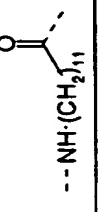
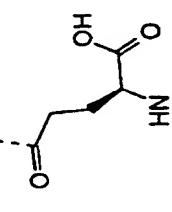
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7		Fmoc-Gln(Trt)	p
8		Fmoc-Gly	p
9		Fmoc-His(Trt)	p
10		Fmoc-Ile	p
11		Fmoc-Leu	p
12		Fmoc-Lys(Boc)	p
13		Fmoc-Met	p

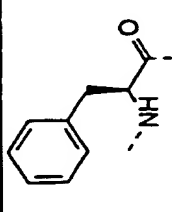
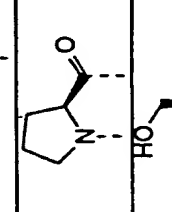
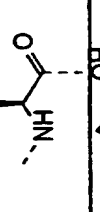
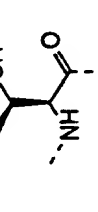
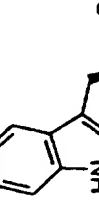
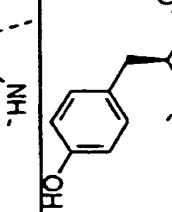
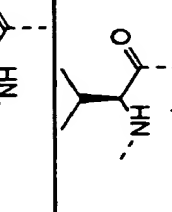
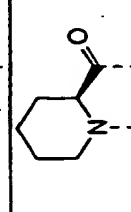
Table 4

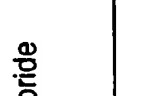
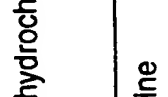
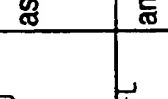
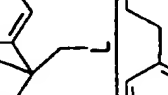

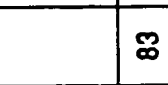



The following chemical compounds are used for the synthesis of the libraries in Examples 2 and 3:

L or "----" = link

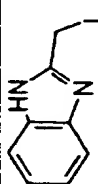
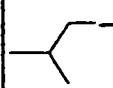
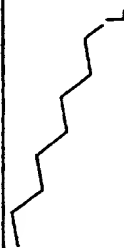





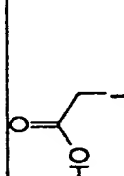
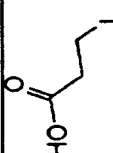


No.	Building Block	Reagent	Link
1		Fmoc-Ala x H <sub>2</sub> O	p
2		Fmoc-Arg(Pmc)	p
3		Fmoc-Asn(Trt)	p
4		Fmoc-Asp(OBt)	p
5		Fmoc-Cys(Trt)	p

53		Fmoc-Pen(Acm)	p
54		Fmoc-D-Pen(Acm)	p
55		Fmoc-β-Ala 9 g	p
56		Fmoc-g aminobutyric acid	p
58		Fmoc-C6-acid	p
60		Fmoc-C8-acid	p
63		Fmoc-C12-acid	p
66		Fmoc-O(But)-protected	p

14		Fmoc-Phe	p
15		Fmoc-Pro	p
16		Fmoc-Ser(But)	p
17		Fmoc-Thr(But)	p
18		Fmoc-Trp(Boc)	p
19		Fmoc-Tyr(But)	p
20		Fmoc-Val	p
51		Fmoc-homo-Pro	p

<b>77</b>		<b>77a amine 77b 2-picolyl chloride HCl</b>	a
<b>78</b>		amine	a
<b>79</b>		amine	a
<b>80</b>		amine	a
<b>81</b>		amine as hydrochloride	as
<b>82</b>		amine as hydrochloride	as
<b>83</b>		amine	a
<b>84</b>		amine	a
<b>85</b>		amine as hydrochloride	as

69		Fmoc (as amine BB 178)	p
70		amine	a
71		amine	a
72		amine	a
73		amine	a
74		amine	a
75		amine	a
76		76a amine; contains debenzyl 76b	a

97		amine as hydrochloride	as
99		amine	a
100		amine 70 g	a
101		amine 4 g	a
102		amine 50 g	a
103		amine as hydrochloride Boc-protected, 4 g	as
104		amine as hydrochloride Boc-protected, 8 g	as
105		amine as hydrochloride Boc-protected, 6 g	as
106		amine as hydrochloride OBu <sup>t</sup> -protected, 3.5 g	as
107		amine as hydrochloride OBu <sup>t</sup> -protected	as
108		amine 100 g	a
109		amine 100 g	a

86		amine	a
87		amine	a
88		amine	a
89		amine (But)-protected	a
90		amine	a
91		amine	a
92		amine as hydrochloride	as
94		amine as hydrochloride	as
95		amine	a

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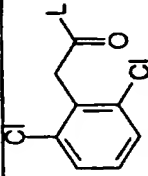
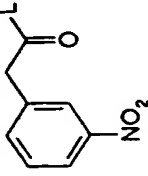
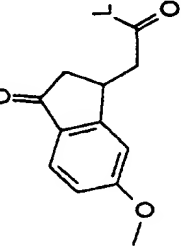
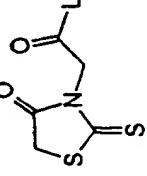
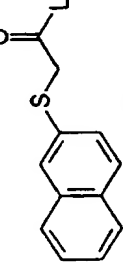
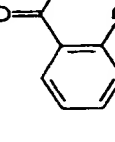
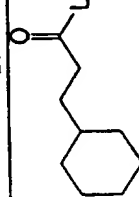
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135			as
143		amine 10 g	a
144		amine	a
145		amine	a

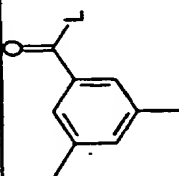

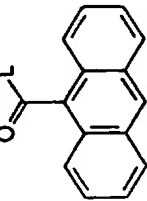
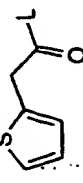
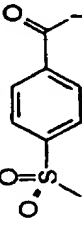
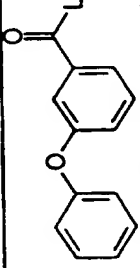
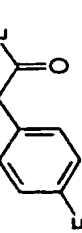
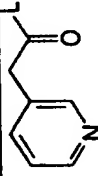
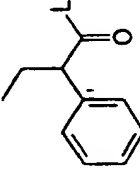
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112		amine 80 g	a
113		5 g	a
115		amine 40 g	a
116		amine 40 g	a
117		117a amine 80 g 117b chloride	a
118		amine 50 g	a
119		amine 100 g	a

320		acid	
321		acid	
322		acid	
323		acid	
325		acid	
327		acid	
328		acid	
329		acid	

146		amine	a
147		amine (Boc)-protected	a
148		amine (Boc)-protected	a
149		amine	as
150		amine	as
151		amine	as
319		acid	

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341		acid	
344		acid	
392		acid	
398		acid	
399		acid	
401		acid	
402		acid	

330		acid	
331		acid	
332		acid	
334		acid	
335		acid	
336		acid	
338		acid	
339		acid	
340		acid	



510		acid	
511		sulfonyl chloride	
512		sulfonyl chloride BB 516= ortho	
514		sulfonyl chloride	
515		sulfonyl chloride	
517		sulfonyl chloride	
518		sulfonyl chloride	
519		sulfonyl chloride	

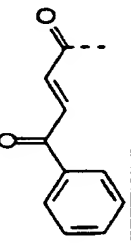
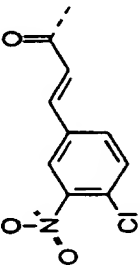
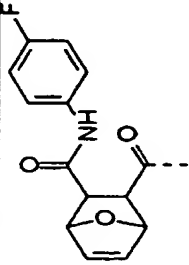
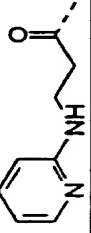
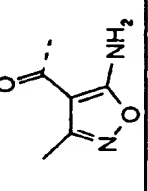
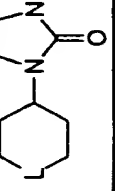
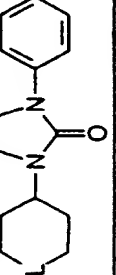
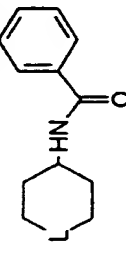
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502		502a acid 502b anhydride 502c acid chloride	
503		acid-OBut	
504		sulfonyl chloride	
505		505a sulfonyl chloride 505b anhydride	
506		acid	
507		sulfonyl chloride	
508			
509		509a acid 509b chloride	

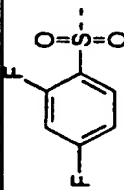
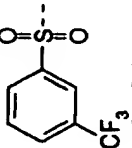
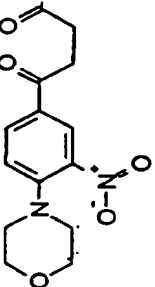
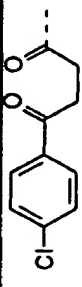
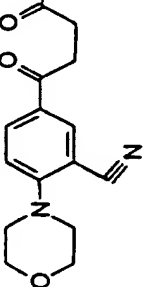
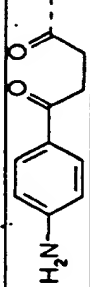
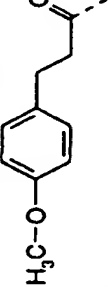
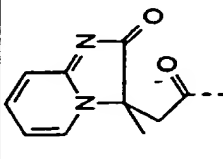
- 40 -

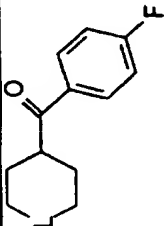
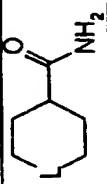

530		sulfonyl chloride	
531		sulfonyl chloride	
532		sulfonyl chloride	
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537		sulfonyl chloride	
538		sulfonyl chloride	

521		sulfonyl chloride	
523		sulfonyl chloride	
524		sulfonyl chloride	
525		sulfonyl chloride	
526		sulfonyl chloride	
527		sulfonyl chloride	
528		sulfonyl chloride	
529		sulfonyl chloride	

SUBSTITUTE SHEET (RULE 26)

547		acid	
548		acid	
549		acid	
550		acid	
551		acid	
552		amine	a
553		amine	a
554		amine	a

539		sulfonyl chloride	
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542		acid	
543		acid	
544		acid	
545		acid	
546		acid	

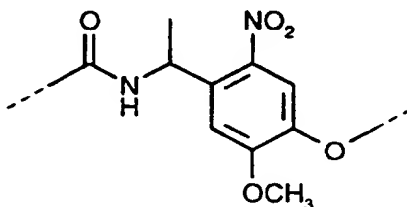
555	 <chem>O=C1CCCCC1C(=O)c2ccc(F)cc2</chem>	amine	a
556	 <chem>NC(=O)C1CCCCC1C(=O)N2CCCC2</chem>	amine	a
557	 <chem>C1CCOCCO1</chem>	amine	a

SUBSTITUTE SHEET (RULE 26)

What is claimed is:

1. A library consisting of a plurality of different units each consisting of a solid or semisolid carrier, a synthetic ligand and an information structure (tag) by means of which the building blocks of the ligand can be identified, wherein
  - a) each carrier unit carries only one type of ligand, and
  - b) each ligand is clearly identified by the presence or absence of one or more elements or element salts.
2. A library according to claim 1, wherein the element salts are non-covalently bonded.
3. A library according to claim 1, wherein each unit of the ligand is clearly coded several times.
4. A library according to claim 1, wherein each of the element salts contains one or more element ions having an atomic mass of more than 80.
5. A library according to claim 1, wherein the elements or element salts are used in the form of water-soluble salts or complexes.
6. A library according to claim 1, wherein the element salts contain Rb, Sr, Y, Zr, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Os, Ir, Pt, Tl, Pb, Bi, Th, W or U.
7. A library according to claim 1, wherein nitrates, hydroxides or chlorides of the element salts are used.
8. A library according to claim 1, wherein the ligand is bound to the carrier *via* a cleavable linker.
9. A library according to claim 1, wherein the ligand is bound to the carrier *via* a linker that is cleavable under basic conditions, under acid conditions, by photolysis, by oxidation, by reduction or by Pd<sup>0</sup>.
10. A library according to claim 1, wherein the ligand is bound to the carrier *via* a linker that is cleavable under basic conditions.

11. A library according to claim 8, wherein there is used as linker a p-hydroxymethylbenzoic acid, 4-hydroxymethylphenylacetic acid, benzhydrylamino, hydroxy-crotonyl-aminomethyl, allyl, 3-nitro-4-hydroxymethylbenzoic acid, p-nitrobenzhydrylamine or 4-[4,4'-bis(methylsulfinyl)-2-oxy-benzhydrylamino]butyric acid linker, a disulfide linker or a linker having the following formula



12. A library according to claim 8, wherein the linker is 4-hydroxymethylbenzoic acid.

13. A library according to claim 1, wherein the solid or semisolid carrier is a polymer resin.

14. A library according to claim 1, wherein the polymer resin contains originally free amino, ether or hydroxyl groups.

15. A process for the production of a library according to claim 1, which comprises adding during the synthesis of a ligand, before, after or during a synthesis step that is to be coded, a combination of elements or element salts that codes for that synthesis step.

16. A process according to claim 15, wherein the element salt is not covalently bonded.

17. A process according to claim 15, wherein the element salt is added in the form of a water-soluble salt or complex.

18. A process according to claim 15, wherein the element salt is added in the form of a nitrate, hydroxide or chloride.

19. A process for the production of a library according to claim 15, which comprises

- a) optionally binding a linker for the ligand to the solid or semisolid carrier;
- b) optionally binding non-variable building blocks of the ligand;
- c) dividing the solid or semisolid carriers into portions for the variable building blocks of the ligand;

- d) in each portion separately, making further modifications to the ligand or attaching another of the variable building blocks possible at that position of the ligand and, before, after or during the synthesis of the ligand, adding a combination of element salts that codes for each synthesis step;
- e) mixing the portions;
- f) optionally binding non-variable building blocks of the ligand or carrying out reactions that are not to be coded;
- g) repeating steps b) to f) until the variable portion of the ligand has been fully synthesised; and
- h) optionally binding other non-variable building blocks to the ligand.

20. A process according to claim 15, wherein after the addition of the element salts the pH value of the solution is adjusted to acid and then to neutral or basic.

21. A process according to claim 15, wherein the reactive side chains of the building blocks of the ligand that are not intended to participate in the reactions are protected using protecting groups.

22. A process according to claim 15, wherein the reactive side chains of the building blocks of the ligand that are not intended to participate in the reactions are protected using protecting groups that are cleavable under acid conditions.

23. A process according to claim 15, wherein the reactive side chains of the building blocks of the ligand that are not intended to participate in the reactions are protected using penta-methylchromansulfonyl (PMC), Mtr or protecting groups of the tert-butyl type.

24. A process for finding structures, wherein the acceptor to be investigated is added to a library according to claim 1 and the constituents of the library that bind the acceptor are identified.

25. A process according to claim 24, wherein the acceptor is a receptor, a transport protein, an antibody, an enzyme, or a fragment thereof.

26. A process according to claim 24, wherein the acceptor carries an identifiable group.

27. A process according to claim 24, wherein the identifiable group is a fluorescent, chemoluminescent or radioactive group, avidin, biotin, a reporter enzyme or an immunologically detectable group.
28. A process according to claim 24, wherein the identifiable group is a fluorescent group.
29. A process according to claim 24, wherein the library according to claim 1 is mixed with an acceptor that carries a fluorescent group and washed, and the constituents of the library that exhibit fluorescence are identified.
30. A process according to claim 24, wherein the ligands are separated from the isolated carriers and again subjected to a test.
31. A process according to claim 24, wherein the identified constituents (beads) are isolated and the composition of the ligands bound thereto is identified by the presence of specific elements or element ions.
32. A process according to claim 31, wherein the elements or element ions present are identified by means of total reflection X-ray fluorescence spectrometry (TRXF), neutron activation with subsequent gamma spectrometry, or by mass spectrometry.
33. A process according to claim 30, wherein the elements or element ions present are identified by means of inductively coupled plasma-mass spectrometry (ICP-MS).



## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K1/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, no. 23, 8 November 1994, WASHINGTON US, pages 10779-10785, XP002007085 K.D. JANDA: "Tagged versus untagged libraries: Methods for the generation and screening of combinatorial chemical libraries" see page 10782, left-hand column, paragraph 5 - page 10784, right-hand column, paragraph 2 ---	1,4-10, 15,19,24
A	WO,A,94 08051 (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); OH) 14 April 1994 see page 52, line 27 - page 53, line 32; claims; examples --- -/--	1,4-10, 15,19,24

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
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Date of the actual completion of the international search

28 June 1996

Date of mailing of the international search report

23.07.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Fuhr, C

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 20242 (SCRIPPS RESEARCH INST ;LERNER RICHARD (US); JANDA KIM (US); BRENNE) 14 October 1993 see page 12, line 5 - page 17, line 23; claims; examples	1,4-10, 15,19,24
P,A	--- CHEMICAL ABSTRACTS, vol. 124, 1996 Columbus, Ohio, US; abstract no. 344060, FELDER, EDUARD R. ET AL: "A new combination of protecting groups and links for encoded synthetic libraries suited for consecutive tests on the solid phase and in solution" XP002007086 see abstract & MOL. DIVERSITY (1996), 1(2), 109-12 CODEN: MODIF4;ISSN: 1381-1991, 1996,	1,11-15, 19,24
P,A	--- WO,A,95 16209 (CIBA GEIGY AG ;FELDER EDUARD (CH); RINK HANS (CH); MATTHEWS IAN TI) 15 June 1995 see the whole document	1,15,19, 24
P,A	--- WO,A,95 28640 (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); WI) 26 October 1995 see page 12, line 34 - page 15, line 17; claims -----	1,15,19, 24

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/01154

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		CA-A-	2143848	14-04-94
		EP-A-	0665897	09-08-95
		HU-A-	72495	28-05-96
		NO-A-	951230	30-03-95
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Form PCT/ISA/210 (patent family annex) (July 1992)

